Effects of Antipsychotic Drugs on the Expression of Synaptic Proteins and Dendritic Outgrowth in Hippocampal Neuronal Cultures

SUNG WOO PARK,1 CHAN HONG LEE,1 HYE YEON CHO,1 MI KYOUNG SEO,1,2 JUNG GOO LEE,1,2,3 BONG JU LEE,1,3 WONGI SEOL,1 BAIK SEOK KEE,3 AND YOUNG HOON KIM1,2,3*

1 Paik Institute for Clinical Research, Inje University, Busan, Republic of Korea
2 Department of Psychiatry, School of Medicine, Inje University, Busan, Republic of Korea
3 Department of Psychiatry, Haeundae Paik Hospital, Inje University, Busan, Republic of Korea
4 InAm Neuroscience Research Center, Wonkwang University, Sanbon Hospital, Gunpo, Republic of Korea
5 Department of Psychiatry, School of Medicine, Chungang University, Seoul, Republic of Korea

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ABSTRACT Recent evidence has suggested that atypical antipsychotic drugs regulate synaptic plasticity. We investigated whether some atypical antipsychotic drugs (olanzapine, aripiprazole, quetiapine, and ziprasidone) altered the expression of synapse-associated proteins in rat hippocampal neuronal cultures under toxic conditions induced by B27 deprivation. A typical antipsychotic, haloperidol, was used for comparison. We measured changes in the expression of various synaptic proteins including postsynaptic density protein-95 (PSD-95), brain-derived neurotrophic factor (BDNF), and synaptophysin (SYP). Then we examined whether these drugs affected the dendritic morphology of hippocampal neurons. We found that olanzapine, aripiprazole, and quetiapine, but not haloperidol, significantly hindered the B27 deprivation-induced decrease in the levels of these synaptic proteins. Ziprasidone did not affect PSD-95 or BDNF levels, but significantly increased the levels of SYP under B27 deprivation conditions. Moreover, olanzapine and aripiprazole individually significantly increased the levels of PSD-95 and BDNF, respectively, even under normal conditions, whereas haloperidol increased the total outgrowth of hippocampal dendrites via PI3K signaling, whereas haloperidol had no effect in this regard. Together, these results suggest that the up-regulation of synaptic proteins and dendritic outgrowth may represent key effects of some atypical antipsychotic drugs but that haloperidol may be associated with distinct actions. Synapse 00:000–000, 2013. © 2013 Wiley Periodicals, Inc.

INTRODUCTION

Schizophrenia is a severe psychiatric illness characterized by positive, negative, and cognitive symptoms (Kinon and Lieberman, 1996). Although the underlying causes of the disorder remain largely unknown, many studies have proposed that schizophrenia may be attributable to abnormalities in brain circuitry that cause reduced and aberrant connectivity in various brain areas (Giaquinta et al., 2005; Hashimoto et al., 2005; Mendrek et al., 2005). In particular, several postmortem studies have noted decreased expression of genes responsible for synaptic proteins in the hippocampus and other regions, suggesting impairment or reduction in synaptic connectivity in schizophrenia (Eastwood and Harrison, 2005; Eastwood et al., 2001). Additionally, these neuronal alterations might underpin the cognitive deficits observed in schizophrenic patients.

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Correspondence to: Young Hoon Kim, M.D., Ph.D, Department of Psychiatry, Inje University Haeundae Paik Hospital, 1435, Jwa-dong, Haeundae-gu, Busan 612-030, Republic of Korea. E-mail: neuro109@hanmail.net

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Synapse dependent synaptic regulation (Yoshii and Constantine-Paton, 2010). BDNF has been implicated in activity-driven synapse stabilization (Ehrlich et al., 2008). Appropriate levels of PSD-95 are required for spine formation and synaptogenesis (Karson et al., 1999). PSD-95 may also be relevant to the pathophysiology of schizophrenia (Toro and Deakin, 2005). The postsynaptic density protein PSD-95 is located largely in dendritic spines and plays a critical role in regulating dendritic spine size and shape (Ehrlich et al., 2007; Han and Kim, 2008) by acting as a scaffolding protein that regulates the clustering of gluta-mate receptors in the dendritic spine (Han and Kim, 2008). Appropriate levels of PSD-95 are required for synaptic maturation, strengthening, plasticity, and activity-driven synapse stabilization (Ehrlich et al., 2007). PSD-95 overexpression is associated with increases in the size of excitatory synapses but reductions in the number of inhibitory synaptic contacts (Prange et al., 2004). PSD-95 may also be relevant to the pathophysiology of schizophrenia (Toro and Deakin, 2005).

Postsynaptic brain-derived neurotrophic factor (BDNF), the most abundant neurotrophin in the brain, contributes to axonal branching, dendritic differentiation, and connectivity among neurons (Ji et al., 2005; Lessmann et al., 2003; Poo, 2001). Numerous studies have reported BDNF-induced changes in dendritic spine density and morphology in various neuronal populations (Shen and Cowan, 2010). BDNF has been implicated in activity-dependent synaptic regulation (Yoshii and Constantine-Paton, 2010). For example, it has been reported that BDNF induces PSD-95 trafficking to dendrites via PI3K/Akt signaling after NMDA activation (Yoshii and Constantine-Paton, 2007). BDNF and its receptor, TrkB, are also required for NMDA-depend-ent long-term potentiation (Kovalchuk et al., 2002; Pang et al., 2004). BDNF is also activated at glutamatergic synapses, increases AMPA receptor currents and induces the maturation of inhibitory neurons (Huang et al., 1999; Pang et al., 2004; Poo, 2001). Changes in BDNF levels have been implicated in both the etiology of schizophrenia and the action of antipsychotic drugs (Bai et al., 2003; Durany and Thome, 2004; Parikh et al., 2004; Park et al., 2006, 2009a,b).

Synaptophysin (SYP), the major integral mem-brane protein of presynaptic vesicles, is required for vesicle formation and exocytosis (Valtorta et al., 2004) and is widely used as a marker for synapse activity (Chambers et al., 2005). SYP plays a regulatory role in activity-dependent synapse formation in cultures of hippocampal neurons (Tarsa and Goda, 2002). Thus, increased SYP expression may reflect increased synaptic activity, density, and vesicles, indicating the improved function of synapses. Loss of SYP in the hippocampus correlates with the cognitive decline associated with Alzheimer’s disease, reflecting its role in cognitive function (Sze et al., 1997). Postmortem studies of patients with schizophrenia have shown decreased expression of SYP in the hippocampus (Vawter et al., 1999) and prefrontal cortex (Karson et al., 1999).

The synapse-associated proteins described above collectively play crucial roles in synapse formation and synaptic plasticity. We propose that atypical antipsychotic drugs may act as key regulators of these proteins. Changes in the expression of synap-tic proteins induced by atypical antipsychotic drugs may be more apparent when measured under reduced synaptic protein levels. We found previ-ously that serum deprivation in SH-SY5Y cell cul-tures decreased the levels of neuroprotective proteins, such as Bel-2 (Kim et al., 2008). Thus, we used a model of toxicity, a procedure that omits B27 in primary culture of the hippocampus, an area particularly relevant to cognitive processes, thereby causing cell death (Bastianetto et al., 2006). In this study, we investigated the expression of synapse-associated proteins under conditions in which rat primary hippocampal cells were and were not subjected to B27 deprivation to assess whether atypical antipsychotic drugs (olanzapine, clozapine, quetiapine, aripiprazole, and ziprasidone) or the typical antipsychotic drug haloperidol affected the expression of these proteins. Next, we investigated whether these drugs affected the dendritic morphology of hippocampal neurons.
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MATERIALS AND METHODS
Drugs and reagents
Neurobasal medium, fetal bovine serum (FBS), horse serum (HS), B27 supplement, L-glutamine, and penicillin–streptomycin were purchased from Invitrogen (Carlsbad, CA), and trypsin and haloperidol were from Sigma (St. Louis, MO). Olanzapine was supplied by Lilly Research (Indianapolis, IN), quetiapine by AstraZeneca (London, UK), aripiprazole by Otsuka Pharmaceutical (Tokushima, Japan), and ziprasidone by Pfizer (NY). Antibodies used for the Western blotting were purchased from the following sources: anti-SYP (sc-7568), anti-BDNF (sc-546), and anti-goat and anti-rabbit IgG-horseradish peroxidase conjugates from Santa Cruz Biotechnology (Santa Cruz, CA), anti-α-tubulin from Sigma; anti-PSD95 (AB9634) from Millipore (Temecula, CA), and ECL anti-mouse IgG-horseradish–peroxidase-linked species-specific whole antibodies from Amersham Pharmacia (Little Chalfont, Buckinghamshire, UK). Antibodies used for immunostaining were purchased from the following sources: antimicrotubule-associated protein 2 (MAP-2) from Millipore and Alexa Fluor 568 goat antimouse IgG and Hoechst 33258 from Invitrogen. The PI3K inhibitor LY294002 was purchased from Cell Signaling Technology (Beverly, MA).

Primary hippocampal cell cultures
All animal manipulations were performed in accordance with the animal-care guidelines of the US National Institutes of Health (NIH publication no. 23–85, revised 1996) and the Korean Academy of Medical Science. This experiment was approved by the Committee for Animal Experimentation and the Institutional Animal Laboratory Review Board of Inje Medical College (approval no. 2009-36).

Primary cultures of hippocampal neurons were prepared from fetal brains (embryonic day 17; E17) obtained from Sprague–Dawley rats (Orient Bio, Gyeonggi-Do, Korea) in a manner similar to that developed by Kaech and Banker (2006). Briefly, brains were exposed, and the hippocampi were carefully removed and dispersed in neurobasal medium containing 0.03% trypsin for 20 min at 37°C (5% CO2). Cells were suspended in a neurobasal medium including 1% FBS, 1% HS, 2% serum-free growth medium B27 (components: biotin, α-tocopherol acetate, α-tocopherol, vitamin A, bovine serum albumin, catalase, insulin, transferrin, superoxide dismutase, corticosterone, galactose, ethanolamine, glutathione, carnitine, linoleic acid, linolenic acid, progesterone, putrescine, selenium, and triodo-L-thyronine), 0.25% L-glutamine, and 50 U/mL penicillin–streptomycin. For Western blotting, neurons were plated in 6-well plates coated with poly-L-lysine at a density of 2 × 10⁴ per well. For the neurite assay, neurons were plated in 12-well dishes at a density of 2 × 10⁵ per well. They were grown under the above condition (normal condition) for 5 days (for neurite assay) or 10 days (for Western blotting). B27 has been demonstrated to facilitate optimal growth and long-term survival of rat embryonic hippocampal neurons. Our preliminary experiment showed that hippocampal cells were reduced by about 32% under B27-free conditions (data not shown). After incubation for 5 or 10 days, the cells were treated with antipsychotic drugs in the presence or absence of B27 for 4 days (for Western blotting) or under normal condition for 5 days (for the neurite assay) and harvested for further analysis. The culture media and drugs were changed every 2 days.

Drug treatment
Antipsychotic drugs (10 mM) were dissolved in dimethyl sulfoxide (DMSO). The solutions were diluted to various concentrations (final concentration of 1% DMSO) with neurobasal medium before use. For Western blotting and the neurite assay, cells were cultured for 4 and 5 days, respectively, with olanzapine (10, 50, and 100 μM), quetiapine (0.1, 1, and 10 μM), aripiprazole (0.1, 1, and 10 μM), ziprasidone (0.1, 1, and 10 μM), or haloperidol (0.1, 1, and 10 μM) in the presence or absence of B27. Control cells were cultured without antipsychotics under normal condition. The drug concentrations used in this study were based on the preliminary studies showing that lower concentrations (<0.1 μM) had not effects on key proteins [Akt or extracellular signal-regulated kinase (ERK)] responsible for the activation of signaling pathways regulating neurite outgrowth and neuronal differentiation, and higher concentrations reduced the viability of hippocampal cells. Additionally, Lu and Dwyer, (2005) reported a similar narrow concentration range for positive effects of atypical antipsychotic drugs on neuronal cells.

Western blot analysis
The cells were washed twice with ice-cold phosphate-buffered saline (PBS, pH 7.4). Lysis buffer [20 mM Tris-HCl, 137 mM NaCl, 10% glycerol, 1% Nonidet P-40, 0.1% sodium dodecyl sulfate (SDS), 0.5% sodium deoxycholate, 2 mM EDTA, and one tablet complete protease inhibitor (Roche, Laval, Quebec, Canada), pH 8.0] was added, and the lysates were centrifuged (1000×g, 15 min, 4°C), and the supernatants were boiled in lysis buffer. Equal amounts of protein (20 μg) derived from the cell extracts under each treatment condition were separated on SDS-polyacrylamide gels and transferred electrophoretically onto polyvinylidene fluoride membranes. The blots were blocked by incubation in 5% (w/v) non-fat milk in Tris-buffered saline (TBS, pH 8.0) with 0.15% Tween 20 (TBS-T) for 1 h. After incubation with a
primary antibody (anti-PSD-95, 1:1000; anti-BDNF, 1:1000; anti-SYP, 1:1000; and anti-α-tubulin, 1:2000) in TBS-T at 4°C overnight, the membranes were washed three times in TBS-T for 10 min. The membranes were subsequently incubated for 1 h in TBS-T containing the horseradish peroxidase-conjugated secondary antibody (anti-SYP, 1:5000; goat-anti-rabbit IgG for anti-PSD95, 1:2000 and anti-BDNF, 1:2000; and anti-mouse IgG for anti-α-tubulin, 1:10000). Immunoreactive bands were visualized and quantified using ECL+ Western blotting reagents, with chemiluminescence detected using the Las-3000 Image Reader (Fuji Film, Tokyo, Japan) software. The amount of protein was normalized based on β-tubulin, a dendritic marker, as follows. Cells were fixed for 20 min at room temperature using 4% paraformaldehyde; they were then permeabilized with 0.1% Triton X-100 and blocked with 4% bovine serum albumin in PBS for 2 h to reduce nonspecific binding. Cells were incubated with the anti-MAP-2 antibody diluted 1/1000 in PBS for 2 h. Alexa Fluor 568 goat anti-mouse IgG was used as a secondary antibody, and Hoechst 33258 was used for nuclear staining. Stained cells were mounted on cover glasses and observed using a fluorescent microscope (Olympus, Tokyo, Japan). For the neurite analysis, two independent experiments for each sample were performed and five fields were randomly selected from each sample. The images were captured through an automatic FISH (Fluorescence in situ hybridization) system with an Olympus BX51 (Olympus, Japan) by a person blinded to their identities. Photomicrographs were cropped and adjusted for brightness and contrast with Adobe Photoshop CS3. At least 300–400 cells were analyzed using MetaMorph software, version 6.1, an automated image-analysis program (Molecular Devices, Downingtown, PA, US; Klimaschewski et al., 2002).

Statistical analysis

For Western blotting and the neurite assay, we obtained samples from three experiments and two independent experiments, respectively. Western blotting was performed two or three times for each sample. The three final values obtained from the three independent maps were used for quantification and statistical analysis. In the neurite assay, 300–400 cells obtained from five randomly picked areas were analyzed from each sample. Cells obtained from one typical sample out of two similar samples were included for quantification and statistical analysis. All statistical analysis were conducted using one-way ANOVA followed by Scheffe’s test. P-values ≤ 0.05 were deemed to indicate statistical significance.

RESULTS

Effects of antipsychotic drugs on postsynaptic proteins PSD-95 and BDNF and presynaptic protein SYP

Antipsychotic drug-induced changes in the expression of the postsynaptic proteins PSD-95 and BDNF and presynaptic protein SYP were measured in rat hippocampal neurons maintained separately in media with or without B27. Olanzapine and four other antipsychotic drugs (aripiprazole, quetiapine, ziprasidone, and haloperidol) were added at concentrations of 10–100 μM and 0.1–10 μM, respectively, for 4 days. B27 deprivation reduced the levels of PSD-95, BDNF, and SYP expression significantly, to ∼ 60.9, 61.3, and 51.2% of control levels, respectively, (P < 0.05 or P < 0.01, Figs. 1).

Olanzapine, aripiprazole, and quetiapine attenuated the B27 deprivation-induced decrease in PSD-95 protein in a concentration-dependent manner (F = 88.949, P = 0.003 for olanzapine; F = 88.499, P = 0.003 for aripiprazole; F = 72.974, P = 0.001 for quetiapine; Figs. 1A–1C). Moreover, high concentrations of olanzapine and aripiprazole increased the PSD-95 level significantly, by 30%, under normal conditions (F = 88.949, P = 0.003 for olanzapine; F = 88.499, P = 0.003 for aripiprazole). In contrast, ziprasidone and haloperidol had no effect on the hippocampal levels of PSD-95 under B27-deprivation conditions (F = 122.132, P = 0.044, Fig. 1E).

Olanzapine and aripiprazole increased the BDNF protein level in hippocampal cells with and without B27 in a concentration-dependent manner (F = 60.668, P < 0.001 for olanzapine; F = 56.291, P = 0.014 for aripiprazole Figs. 2A and 2B). At concentrations up to 10 μM, quetiapine produced this effect only under B27 deprivation conditions (F = 65.429, P < 0.001, Fig. 2C). Neither ziprasidone nor haloperidol changed the BDNF level in hippocampal neurons (Figs. 2D and 2E).

Olanzapine, aripiprazole, quetiapine, and ziprasidone increased SYP protein levels in the hippocampal neurons significantly, by 30%, under normal conditions (F = 88.949, P < 0.001, Figs. 3A–3D) but not in normal cultures. In contrast, the levels of this presynaptic protein in the hippocampal neurons were unchanged by haloperidol (Fig. 3E).

Effects of antipsychotic drugs on dendritic outgrowth

We performed a neurite outgrowth assay to investigate whether antipsychotic drugs regulated dendritic

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morphology in hippocampal neurons. Primary hippocampal cells were incubated for 5 days with different antipsychotic drugs at different concentrations (olanzapine at 100 μM and the other four antipsychotic drugs at 10 μM) that produced maximum effects on the expression of synapse-associated proteins.

Hippocampal cells treated without antipsychotic drugs produced modest dendritic differentiation, with an average dendrite length of around 40 μm (Fig. 4B). These results indicate that all atypical antipsychotic drugs tested increased dendritic outgrowth and branching in hippocampal cells (all P < 0.001, Figs. 4A–4C). This enhancement was most pronounced in olanzapine-treated cells. In contrast, no effect on dendrite outgrowth and branching was observed following haloperidol treatment.

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BDNF is believed to induce neuronal differentiation and synaptogenesis by binding to TrkB receptors and activating phosphatidylinositol 3-kinase (PI3K)/Akt and other pathways (Chambers et al., 2005). We found that atypical antipsychotic drugs increased the levels of BDNF (Fig. 2). To explore a possible relationship between PI3K and the morphological changes induced by these drugs, we examined the effects of the PI3K inhibitor LY294002 on the morphological changes induced in neurons by atypical antipsychotic drugs. LY294002 concentrations ranging from 0.1 to 10 μM had no significant effects on dendritic outgrowth and branching (data not shown). Representative images and data in Figure 5 show that LY294002 (10 μM) inhibited the enhancement of dendritic outgrowth and branching induced by atypical antipsychotic drugs (dendritic outgrowth: \( F = 82.965, \; P < 0.001 \) for olanzapine; \( F = 44.113, \; P < 0.001 \)).

**Fig. 2.** Effects of antipsychotic drugs on the expression of BDNF in hippocampal neurons. Cells were treated with different doses of antipsychotic drugs for 4 days with (+B27) or without (−B27) B27. A: olanzapine, (B) aripiprazole, (C) quetiapine, (D) ziprasidone, and (E) haloperidol. For each condition, three independent experiments were performed. Cell lysates were analyzed by SDS-PAGE and Western blotting with anti-BDNF primary antibodies. A representative image and quantitative analysis normalized to the α-tubulin band are shown. Values are means±SEMs expressed as percentage of values of the control cells (+B27, no drug treatment). *P<0.05 vs. untreated control, **P<0.01 vs. untreated control, *P<0.05 vs. B27-deprived only, **P<0.01 vs. B27-deprived only.
P<0.001 for aripiprazole; F=31.296, P<0.001 for quetiapine; and F=32.757, P<0.001 for ziprasidone, branching: F=93.744, P<0.001 for olanzapine; F=23.656, P<0.001 for aripiprazole; F=19.616, P<0.001 for quetiapine; and F=24.978, P<0.001 for ziprasidone). These data suggest that PI3K activity is required for atypical antipsychotic drugs to enhance hippocampal dendritogenesis.

Fig. 3. Effects of antipsychotic drugs on the expression of SYP in hippocampal neurons. Cells were treated with different doses of antipsychotic drugs for 4 days with (+B27) or without (−B27) B27. A: olanzapine, (B) aripiprazole, (C) quetiapine, (D) ziprasidone, and (E) haloperidol. For each condition, three independent experiments were performed. Cell lysates were analyzed by SDS-PAGE and Western blotting with anti-SYP primary antibodies. A representative image and quantitative analysis normalized to the α-tubulin band are shown. Values are means±SEMs expressed as percentage of values of the control cells (+B27, no drug treatment). *P<0.05 vs. untreated control, **P<0.01 vs. untreated control, *P<0.05 vs. B27-deprived only, ##P<0.01 vs. B27-deprived only.

**DISCUSSION**

This study is the first report of positive effects of some atypical antipsychotic drugs (olanzapine, aripiprazole, quetiapine, and ziprasidone) on the levels of synapse-associated proteins and dendritic outgrowth in hippocampal neurons. We found that these atypical drugs, but not haloperidol, increased the expression of several proteins associated with synapse...
structure and activity in B27-deprived hippocampal cultures. We also showed the contribution of PI3K signaling to increases in hippocampal outgrowth induced by atypical antipsychotics. However, haloperidol was not associated with these effects.

Olanzapine and aripiprazole increased BDNF and PSD-95 protein levels in hippocampal cultures with and without B27. Quetiapine inhibited the reduction of these proteins, which would have otherwise been induced by B27 deprivation. Ziprasidone had no effects, but haloperidol decreased the levels of PSD-95 under normal conditions. There is considerable evidence that BDNF and PSD-95 may play a direct role in postsynaptic morphogenesis by increasing the number and size of dendritic spines in hippocampal neurons (El-Husseini et al., 2000; Tyler and Pozzo-Miller, 2003). This is supported by other studies demonstrating that BDNF treatment in cultured neurons promotes the growth of spines by regulating the formation of PSD-95-TrkB complexes (Yoshii and Constantine-Paton, 2010), and NMDA receptor-dependent BDNF activation facilitates the rapid transport of PSD-95 to dendritic spines through PI3K signaling (Yoshii and Constantine-Paton, 2007).

Thus, increase in these proteins at glutamatergic synapses may have a beneficial impact on information flow to other neurons. Given the roles of BDNF and PSD-95 in spine formation, the results of this study could suggest that some atypical antipsychotic drugs may induce the promotion of new synapse formation in hippocampal neurons. However, further research examining the density and morphology of synapses is required to confirm this.

Many in vitro and in vivo studies have found that BDNF mRNA and protein are up-regulated by atypical antipsychotics, including olanzapine, clozapine, quetiapine, aripiprazole, and ziprasidone, whereas haloperidol down-regulates or does not affect BDNF levels (Bai et al., 2003; Park et al., 2006, 2009a,b). The transcription factor cAMP response element binding protein (CREB) induces expression of BDNF, which contributes to the stabilization of synaptic plasticity. CREB activity is also elevated following treatment with atypical drugs (Hammonds and Shim, 2009; Park et al., 2009a). Thus, atypical drugs may influence synaptic plasticity via CREB activation.

Several studies have investigated the effects of treatment with antipsychotic drugs on PSD-95. One

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study reported that chronic treatment with haloperidol increased PSD-95 mRNA expression significantly in the rat striatum, consistent with its action at nigrostriatal projections and its propensity to produce motor side effects, but did not affect the hippocampus significantly (Iasevoli et al., 2010). Another study demonstrated that acute administration of haloperidol or olanzapine did not modulate PSD-95 expression in rat forebrains (de Bartolomeis et al., 2002). Little is known about the mechanism of action of antipsychotic drugs on PSD-95, despite its potential importance for understanding the pathophysiology of schizophrenia. Only one reported study has focused on the role of PSD-95 in mediating 5-HT2A receptors, the main site of action of atypical antipsychotic drugs (Abbas et al., 2009). According to this study, the...
absence of PSD-95 decreased the targeting of 5-HT_{2A} receptors, the total expression of 5-HT_{2A} receptors, and 5-HT_{2A}-mediated signaling (p-ERK1/2 and p-GSK-3β). Moreover, the antipsychotic-like action of clozapine was dramatically impaired in PSD-95-null mice. Together with our data, this evidence suggests that up-regulation of PSD-95 expression with atypical antipsychotic drugs may increase synapse formation by modulating 5-HT_{2A}-receptor function.

All atypical antipsychotic drugs tested in this study effectively enhanced dendritogenesis via the PI3K pathway when administered at concentrations that produced maximum effects on BDNF and PSD-95 levels. Although levels of BDNF and PSD-95 were not affected by ziprasidone treatment, enhancement of dendritic outgrowth and branching was observed in ziprasidone-treated hippocampal neurons. This discrepancy may be attributable to the differential effects of different durations of drug treatment (4 days for protein expression vs. 5 days for dendritic outgrowth). The PI3K inhibitor LY294002 did not affect dendritogenesis but did decrease the differentiation induced by atypical antipsychotic drugs, which suggests that PI3K contributes to responses to these drugs. This finding is similar to those of a previous study showing the involvement of the PI3K/Akt pathway in the positive effects of olanzapine, quetiapine, and clozapine on neurite outgrowth in PC12 cells (Lu and Dwyer, 2005). Recent studies have underscored the importance of the PI3K/Akt pathway in schizophrenia (for review, see Zheng et al., 2012); these studies suggest that reduced activity of this signaling could at least partially explain the cognitive impairment, synaptic morphological abnormalities, neuronal atrophy, and dysfunction of neurotransmitter signaling observed in schizophrenia. Moreover, it is thought that reduced levels of Akt may reduce the response of patients to antipsychotic drugs (Zheng et al., 2012). Thus, this signaling pathway appears to be essential for the potentiating effects of olanzapine, aripiprazole, quetiapine, and ziprasidone. However, haloperidol did not influence these effects. Moreover, an important role of PI3K/Akt signaling in the determination of dendritic morphogenesis has been reported. Kumar et al. (2005) showed that PI3K/Akt signaling in rat hippocampal neurons increased dendrite size and dendritic complexity as well as density of dendritic spine via activation of mammalian target of rapamycin (mTOR), which controls new protein synthesis required for synaptic connections that lead to synaptogenesis (Yang et al., 2008). Thus, we hypothesized that some atypical antipsychotic drugs might also play a role in increasing not only dendritic outgrowth but also expression of synaptic proteins via activation of PI3K.

This study revealed that atypical antipsychotic drugs increase SYP expression under B27 deprivation but not under normal conditions. No change was observed in haloperidol-treated hippocampal cells. It has been reported that haloperidol decreased SYP levels in the hippocampus of rats (Eastwood et al., 1997). This discrepancy might be attributable to the difference in experimental paradigms (the dosage, duration of administration, and animals vs. cells) used in the present and previous studies. Only one study on atypical antipsychotic drugs showed that clozapine increased SYP levels in rat frontal cortex (Bragina et al., 2006). SYP plays a central role in the rapid retrieval of synaptic vesicles, which is required for efficient synaptic transmission (Daly et al., 2000). The increased expression of SYP induced by atypical antipsychotic drugs may reflect an increase in the complement of synaptic vesicles, leading to an increased potential for neurotransmitter release (Bragina et al., 2006).

Overall, our results suggest that some atypical antipsychotic drugs, but not typical haloperidol, may mediate synaptic plasticity by modulating synaptic proteins levels and dendritic outgrowth. It may also provide evidence that some atypical antipsychotic drugs induce synaptic reorganization. These effects may contribute to some degree to improvements in cognitive function in patients with schizophrenia.

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